Reference: *Biol. Bull.*, 144: 276–288. (April, 1973)

COMPARISON OF THE PIGMENTARY EFFECTOR TROPINS IN THE EYESTALKS AND ABDOMINAL NERVE CORD OF THE PRAWN *PALAEMONETES VULGARIS* [1]

MILTON FINGERMAN AND CLELMER K. BARTELL

Department of Biology, Tulane University, New Orleans, Louisiana 70118, Department of Biology, Louisiana State University in New Orleans, New Orleans, Louisiana 70122 and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

In 1935 Brown concluded that each of the chromatophoric pigments in the prawn *Palaemonetes vulgaris* was under separate hormonal control. This conclusion was based on his observation that each pigment was capable of movements which were completely independent of those of the other pigments. Perkins (1928) had found a red pigment-concentrating hormone in the eyestalks of this prawn. Later it was shown that the eyestalks also contain a substance that causes dispersion of the melanin in crab melanophores (Brown, 1940; Brown and Scudamore, 1940). *Palaemonetes* itself lacks melanophores. Still later, Brown, Webb and Sandeen (1952) found that this prawn also produces a red pigment-dispersing hormone. The ratio of red pigment-dispersing hormone to red pigment-concentrating hormone appeared to be highest in the abdominal nerve cord. The abdominal nerve cord of *Palaemonetes* also possesses the substance that evokes melanin dispersion in crabs (Fingerman and Couch, 1967). Not only are the erythrophores of *Palaemonetes* controlled by pigment-dispersing and pigment-concentrating substances but also the leucophores (Fingerman, 1970). In the meantime, Kleinholz (1936) found that eyestalk extracts from *Palaemonetes*, in addition to their chromatophorotropic activities, evoked light adaptation of this prawn's distal retinal pigment.

The data of Fingerman and Couch (1967) suggested that the substance in *Palaemonetes* that caused red pigment dispersion in the prawn was not the same substance as that which had been found in the prawn and caused melanin dispersion in the fiddler crab. The portions of the central nervous system which were assayed differed with respect to the ratio of the amounts of melanin dispersion and red pigment dispersion they evoked. In these earlier experiments, however, crude extracts were used and, therefore, no definite conclusion could be drawn concerning that possible identity or non-identity of these substances. The changes in this ratio of red pigment-dispersing activity to melanin-dispersing activity were due to either differences in the relative quantities of a pigment-dispersing substance which acted on both types of chromatophores and antagonistically-acting pigment-concentrating substances among the tissues assayed or, alternatively, the fact that melanin dispersion and red pigment dispersion were caused by different substances.

The main object of the experiments described below is to examine further the

[1] This investigation was supported by Grant GB-27497 from the National Science Foundation.

question of whether red pigment dispersion in the prawn and melanin dispersion in the fiddler crab are caused by the same or different substances from the prawn. The technique of gel chromatography on Bio-Gel P-6 which has been used to separate pigment-dispersing and pigment-concentrating substances of crustaceans from each other was used herein. For example, this technique has allowed the separation of red pigment-dispersing and melanin-dispersing activities from the corresponding pigment-concentrating activities in extracts of eyestalks from the fiddler crab *Uca pugilator* (Fingerman, Bartell and Krasnow, 1971; Fingerman and Fingerman, 1972). Use of this technique would provide us pigment-dispersing material without interference from pigment concentrators and thereby allow resolution of the problem. Furthermore, Fingerman, Krasnow and Fingerman (1971) have suggested that if the melanin-dispersing substance found in *Palaemonetes* is not the same substance as the red pigment-dispersing substance it may be serving as the distal retinal pigment light-adapting hormone of the prawn inasmuch as the prawn which has a high titer of this melanin-dispersing substance in its eyestalks lacks melanophores. In the present experiments this possibility is explored further also.

Materials and Methods

The specimens of the prawn *Palaemonetes vulgaris* were collected in the vicinity of Woods Hole, Massachusetts, by members of the Supply Department of the Marine Biological Laboratory. The fiddler crabs, *Uca pugilator*, were provided by the Gulf Specimen Company of Panacea, Florida.

The system of Hogben and Slome (1931) was used to stage the chromatophores. According to their scheme stage 1 represents maximal pigment concentration, stage 5 maximal dispersion and stages 2, 3 and 4 the intermediate conditions. The chromatophores were staged at the time of injection of the extract being tested, 5, 15 and 30 minutes thereafter and subsequently at 30 minute intervals until the chromatophores had returned to their original stage. The recorded stage values were then used to calculate Standard Integrated Responses (SIR) which are a measure that includes both the amplitude and duration of the response (Fingerman, Rao and Bartell, 1967). With the prawn, the erythrophores and leucophores in the epidermis adhering to the portion of the carapace dorsal to the heart were staged; with the fiddler crab, the melanophores seen through the exoskeleton on the anteroventral surface of the second walking leg on the right side of the animal were staged. Eyestalkless crabs with maximally concentrated melanin were used to assay for the melanin-dispersing substance. Intact crabs in black pans with maximally dispersed melanin were used to assay for a melanin-concentrating substance. Eyestalkless prawns with their white pigment in an intermediate state of dispersion (Fingerman, 1970) were used to assay for white pigment-dispersing and pigment-concentrating substances. These eyestalkless prawns were also used in the assays for the red pigment-concentrating substance because their red pigment became maximally dispersed after their eyestalks had been removed. Prawns having one eyestalk were placed in white containers until their red pigment maximally concentrated and then served in the assays for the red pigment-dispersing substance. Prawns with one eyestalk are more sensitive to this substance than are intact prawns and, therefore, provide a better assay system for it (Brown, Webb

and Sandeen, 1952). The injected dose throughout these experiments was 0.05 ml for both species.

Assays for distal retinal pigment light-adapting activity were performed using the method of Sandeen and Brown (1952). Their technique consists essentially of placing a prawn on the stage of a dissecting microscope and measuring with the aid of an ocular micrometer and transmitted light: (A) the width of the transparent area of the distal portion of the eye in the direction of the long axis of the eyestalk and (B) the distance from the corneal surface to the proximal edge of the pigment spot at the base of the eye on the dorsal surface of the eyestalk. The ratio of A/B is known as the Distal Pigment Index (DPI). Each morning prawns to be used in an assay for the distal retinal pigment light-adapting substance were taken from the stock aquaria and placed in black pans in a darkroom until their distal retinal pigment had become fully dark-adapted. The extracts were then injected with the aid of a dim red lamp while the prawns were still in the photographic darkroom. The prawns were then kept in darkness for one additional hour at the end of which the DPI of each prawn was determined. Control prawns received saline alone.

Gel chromatography of extracts of eyestalks and abdominal nerve cords from the prawn was performed in essentially the same manner as described by Fingerman and Fingerman (1972) with eyestalks of other crustaceans. After either 100 eyestalks or 50 abdominal nerve cords had been extracted in 0.3 ml of 0.065 M sodium chloride, the extract was centrifuged for five minutes at 1815 g and 23–25° C. The supernatant was then applied to the top of a 0.8×31.0 cm column of Bio-Gel P-6, 100–200 mesh (Bio-Rad), prepared with 0.065 M sodium chloride. The void volume of this column was 6.5 ml and its flow rate was 0.5 ml per minute. The active material was eluted with the 0.065 M sodium chloride solution. One ml fractions were collected. To each fraction was added 0.29 ml of 400% crustacean saline (Pantin, 1934) which made the resulting solution so close to the osmotic concentrations of the blood of the prawn and the crab, that coupled with the dilution of the injected material in the blood of the recipients, control specimens showed no significant response in the absence of pigmentary effector activators. Twice as many eyestalks were used as abdominal cords because each prawn had two eyestalks but only one abdominal nerve cord. The prawns of the size used in these experiments had an average weight of 0.57 g. One of their eyestalks averaged 3.0 mg and their abdominal nerve cord averaged 1.3 mg.

In order better to compare the quantities of pigmentary effector tropins in the fractions obtained from the columns, dosage-response curves were obtained by assaying dilutions of the fractions which were most active in evoking melanin dispersion, red pigment dispersion and light adaptation of the distal retinal pigment. Then by use of these curves in which relative concentration was plotted *versus* SIR or DPI we could determine the relative difference in concentration of active material present in different fractions after they had been used in an assay. For example, when fractions evoked different red pigment-dispersing SIR's, examination of the appropriate dosage-response curve would reveal the relative concentration of the red pigment-dispersing substance in each fraction. These dosage-response curves provided a way to compare the quantity of a pigmentary effector tropin in each of these partially purified fractions. With such partially purified fractions one does not have to contend with the complications that occur when

dealing with a mixture of antagonistically-acting substances. Simply comparing SIR's or DPI's does not reveal relative concentrations. For example, an SIR of "4" does not necessarily mean there is twice as much hormone present in one fraction as in a second which evoked an SIR of "2."

Each of these experiments was performed twice. Assays for chromatophore activators were performed on three animals each, those for distal retinal pigment

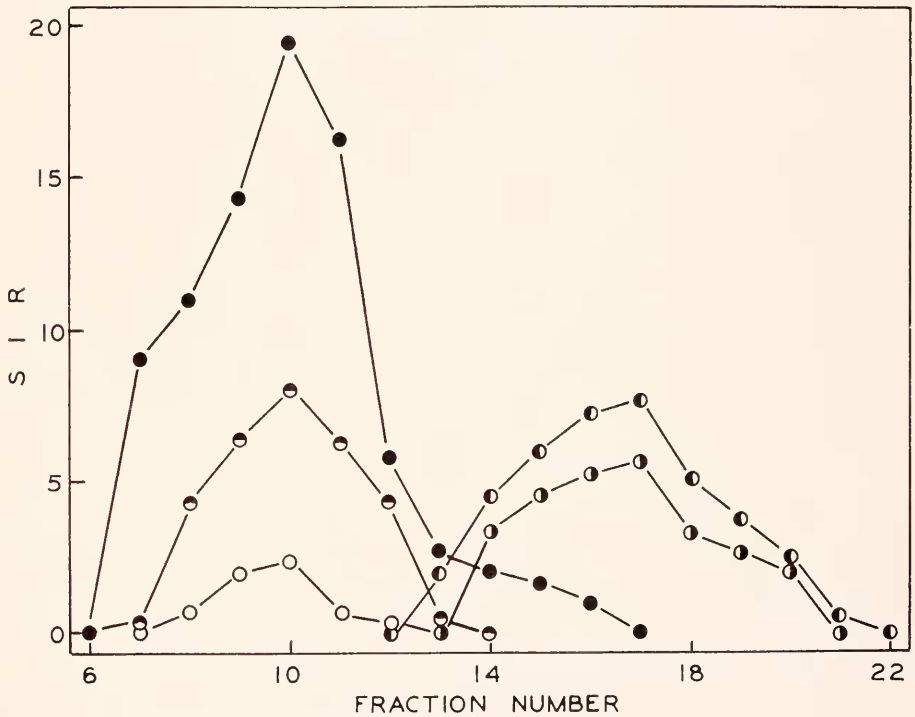

FIGURE 1. The Standard Integrated Responses (SIR) of the melanophores of fiddler crabs and the erythrophores and leucophores of prawns evoked by the fractions obtained by passing extracts of prawn eyestalks through a column of Bio-Gel P-6; dots, melanin-dispersing responses; circles, red pigment-dispersing responses; circles half-filled on top, white pigment-dispersing responses; circles half-filled on left, red pigment-concentrating responses; circles half-filled on right, white pigment-concentrating responses.

light adaptation on seven prawns each. Therefore, the averaged results in the following figures for chromatophoric responses represent the mean for six specimens and for distal retinal pigment light adaptation a mean of 14 specimens.

EXPERIMENTS AND RESULTS

The object of the first set of experiments was to determine the elution profiles of chromatophorotropins from prawn eyestalks and abdominal nerve cords which had been passed through a column of Bio-Gel P-6 and to compare the responses obtained from assays of these partially purified substances with the chromatophoric

responses obtained from assays of crude extracts of these organs. The mean results of the gel chromatographic analysis of the eyestalk extracts are presented in Figure 1. It can be seen there that the largest SIR's were obtained from the fractions that had been assayed for melanin-dispersing activity in eyestalkless fiddler crabs. In addition, red pigment-dispersing, white pigment-dispersing, red pigment-concentrating and white pigment-concentrating activities in the prawn were found. No black pigment-concentrating activity in the fiddler crab was found. All of the pigment-dispersing activities were eluted with an R_f of 0.65 whereas the two pigment-concentrating activities were eluted from the column later and both had an R_f of 0.38; R_f = void volume/elution volume of maximal activity.

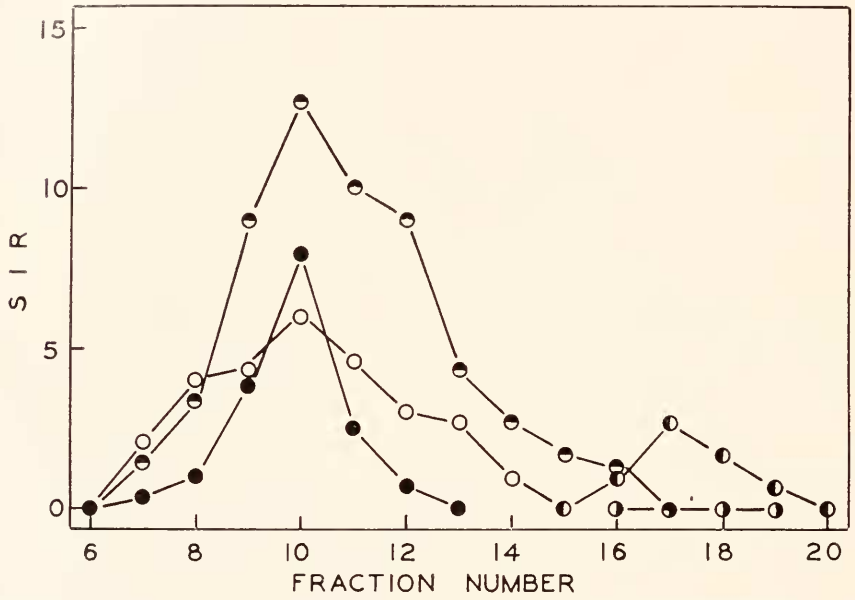

FIGURE 2. The Standard Integrated Responses (SIR) of the melanophores of fiddler crabs and the erythrophores and leucophores of prawns evoked by the fractions obtained by passing extracts of prawn abdominal nerve cords through a column of Bio-Gel P-6. See Figure 1 for key to symbols.

The mean results for the gel chromatographic analysis of the abdominal nerve cord extracts from the prawn are presented in Figure 2. Peaks of red and white pigment-dispersing activities in the prawn and melanin-dispersing activity in the fiddler crab, each having the same elution value (R_f 0.65) as the three pigment-dispersing activities from the eyestalks, were present here also. However, although a small red pigment-concentrating activity was noted (R_f 0.38), separated from the pigment-dispersing activities, neither white pigment concentration in the prawn nor melanin concentration in the fiddler crab was detected with these fractions of the abdominal nerve cords. If this prawn has melanin-concentrating activity in its eyestalks or abdominal nerve cord and white pigment-concentrating activity in its abdominal nerve cord, these activities must be present in very low concentrations

for these assays to have failed to detect them. Comparison of Figures 1 and 2 reveals that there was a considerable difference among the melanin-dispersing and red pigment-dispersing potencies of the eyestalks and abdominal nerve cords. The abdominal nerve cords contained more red pigment-dispersing activity but less melanin-dispersing activity than did the eyestalks. In contrast the relationship of the white and red pigment-dispersing activities to each other was the same in both the eyestalks and abdominal nerve cords, there having been consistently more of the former than the latter.

The crude extracts of the prawn abdominal nerve cord were prepared in a concentration of 1/12 equivalent per dose, prawn eyestalk extracts in both 1/6 and 1/12 equivalent per dose. The SIR's evoked by these extracts are presented in Table I. It can be seen there that injection of the crude eyestalk extracts resulted in a strong melanin-dispersing response in the crab and a strong red-pigment concentrating response in the prawn but no red pigment dispersion occurred in the prawn. In contrast, the abdominal nerve cord extracts evoked very similar SIR's for both melanin dispersion in the crab and red pigment dispersion in the prawn

TABLE I

Standard Integrated Responses (SIR) to crude extracts of eyestalks and abdominal nerve cords of the prawn

Organs extracted	Concentration (organ equivalents per dose)	SIR for melanin dispersion in the fiddler crab	SIR for red pigment dispersion in the prawn	SIR for red pigment concentration in the prawn
Eyestalks	1/6	17.1	0.0	15.2
Eyestalks	1/12	12.5	0.0	11.6
Abdominal nerve cords	1/12	4.9	5.3	0.0

but failed to evoke concentration of the red pigment in the prawn. The lack, for example, of a red pigment-dispersing response with the crude eyestalk extracts but its appearance when the partially purified eyestalk extracts were assayed (Fig. 1) is an example of the sort of antagonism which can occur between pigment-dispersing and pigment-concentrating chromatophorotropins. In the eyestalks there is sufficient red pigment-concentrating hormone to mask completely the red pigment-dispersing substance.

In order to gain further information concerning the relative quantities of the melanin-dispersing and red pigment-dispersing activities in the eyestalks and abdominal nerve cords, fraction 10 of the eyestalks (Fig. 1) which evoked the largest melanin-dispersing response and fraction 10 of the abdominal nerve cords (Fig. 2) which evoked the largest red pigment-dispersing response were diluted with 100% crustacean saline to concentrations of 1/3, 1/9, 1/27 and 1/81 of the original. The dilutions of the eyestalk fraction were then assayed to determine the melanin-dispersing SIR's they evoked and the dilutions of the abdominal nerve cord fraction were similarly assayed to determine their red pigment-dispersing SIR's. The averaged results of these assays were used in the preparation of the dosage-response curves of Figure 3. By use of the data of Figure 3, as described above, we find that the red-pigment dispersing SIR evoked by fraction 10 of the

abdominal nerve cords (Fig. 2) was due to 3.3 times as much active material as was responsible for the red pigment-dispersing SIR obtained with fraction 10 of the eyestalks (Fig. 1). A similar analysis reveals that the melanin-dispersing SIR of fraction 10 of the abdominal nerve cords was due to 0.3 as much melanin-dispersing material as caused the melanin-dispersing SIR of fraction 10 of the eyestalks.

The following experiment was designed to examine the possibility that the hormonal control of the erythrophores is not the same as that of the distal retinal pigment of *Palaemonetes*. These particular chromatophores were chosen for study

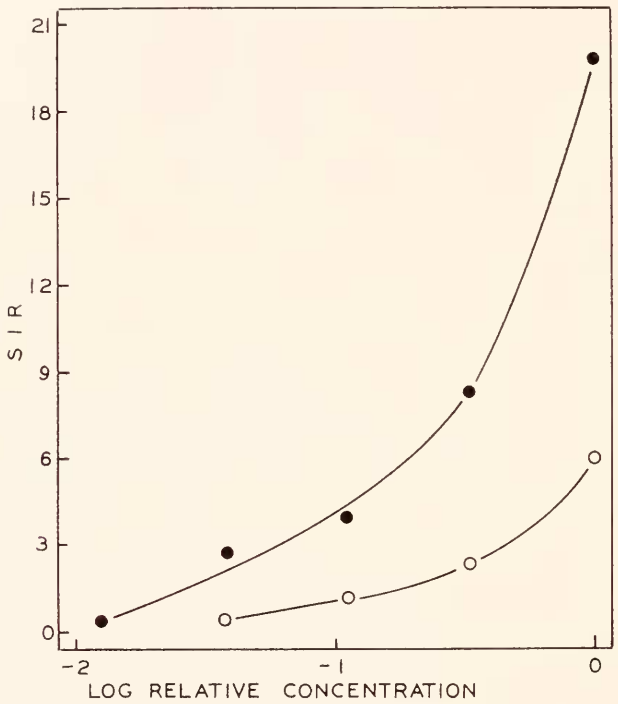

FIGURE 3. Relationships between the Standard Integrated Response (SIR) and the logarithm of the relative concentration of fraction 10 of the prawn eyestalks with respect to melanin dispersion in the fiddler crab (dots) and fraction 10 of the prawn abdominal nerve cords with respect to red pigment dispersion in the prawn (circles).

because they are the predominant type in determing the coloration of this prawn. Twenty intact prawns were evenly divided among two black and two white pans. The prawns in one black and one white pan were then exposed to an illumination of 0.52 lux while the remaining prawns were exposed to an illumination of 1400 lux. One hour later the stages of the erythrophores and the DPI of these prawns were determined. The experiment was repeated and the data averaged. The data revealed that under both light intensities the means stage of the erythrophores of the prawns in the white pans was 1.0 (maximally concentrated) and of those in the black containers 4.8 (nearly maximally dispersed). The mean DPI of the prawns

in the white pans exposed to 0.52 lux was 0.172; in the black pans at 0.52 lux, 0.093; in the white pans at 1400 lux, 0.261; in the black pans at 1400 lux, 0.208. We see from these data that the DPI and erythrophore stages are independent of each other. For example, the DPI of prawns in black pans under an illumination of 1400 lux was larger than the DPI of the prawns in white pans under an illumination of 0.52 lux but smaller than the DPI of the prawns in white pans under an illumination of 1400 lux, yet the red chromatophoric pigment of the prawns in the black pans was nearly maximally dispersed while this pigment was always maximally concentrated in the prawns in the white pans. On the basis of these data, it is valid to conclude that the hormonal control cannot be completely the same for both of these pigmentary effectors.

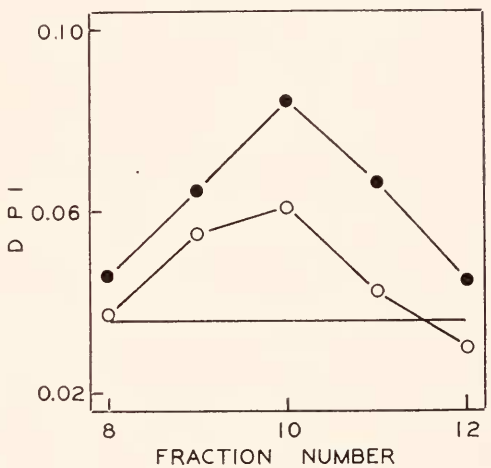

FIGURE 4. The Distal Pigment Indexes (DPI) of prawns which had received injections of fractions of prawn eyestalks (dots) or abdominal nerve cords (circles). The horizontal line at DPI = 0.036 is the mean value of the controls.

The aim of the next experiment was to compare the distal retinal pigment light-adapting potencies of crude extracts of eyestalks and abdominal nerve cords. Prawns that received the equivalent of one-half abdominal nerve cord per dose had a DPI of 0.074 one hour after the extract had been injected whereas prawns that received eyestalk extracts prepared in a concentration of either one-half or one eyestalk equivalent per dose had DPI's one hour after the extracts were injected of 0.168 and 0.219, respectively. The mean value for the controls was 0.036. The crude extracts of the abdominal nerve cords evoked less light adaptation of the distal retinal pigment than did the crude extracts of the eyestalks.

Figure 4 presents the mean results obtained when fractions of the eyestalks and abdominal nerve cords obtained from the Bio-Gel P-6 column were assayed to determine their light-adapting potencies. With each set of fractions, the maximal distal retinal pigment light-adapting activity was obtained with fraction 10 (R_f = 0.65) just as occurred when these fractions were assayed for pigment-dispersing activities on the chromatophores (Figs. 1 and 2). The overall light-adapting re-

sponse to the eyestalk fractions was greater than that evoked by the fractions of the abdominal nerve cords, which is in agreement with the results of the previous experiment where with the crude extracts the eyestalks were more effective than the abdominal nerve cords in evoking a light-adapting response.

The diluted preparations of fraction 10 of the eyestalks which had been used to obtain the melanin-dispersing dosage-response curve of Figure 3 were then assayed to provide data for a dosage-response curve showing the relationship between their distal retinal pigment light-adapting activities (DPI) and relative concentrations (Fig. 5). Using Figure 5 in the same manner as was done to calcu-

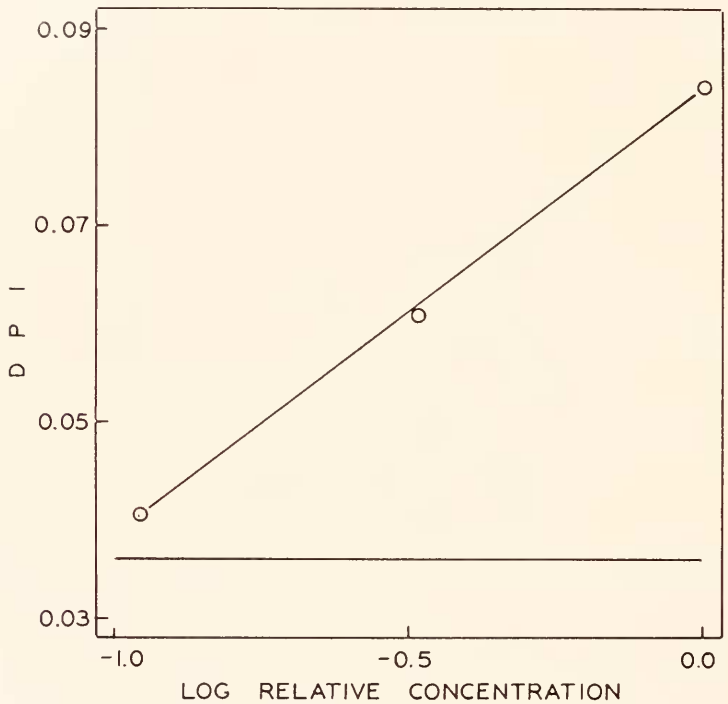

FIGURE 5. Relationship between the Distal Pigment Index (DPI) and the logarithm of the relative concentration of fraction 10 of the prawn eyestalks which was injected. The horizontal line at DPI = 0.036 is the mean value of the controls.

late the relative concentrations of pigment-dispersing material in the fractions of the eyestalks and abdominal nerve cords, we found that fraction 10 of the abdominal nerve cords contained 0.3 as much distal retinal pigment light-adapting substance as did fraction 10 of the eyestalks.

DISCUSSION

These experiments have revealed that the substance in the prawn *Palaemonetes vulgaris* which causes red pigment dispersion and perhaps white pigment dispersion as well is different from the substance also found in this prawn that evokes melanin

dispersion in the fiddler crab *Uca pugilator*. Kleinholz (1970) had chromatographed extracts of eyestalks alone from the prawn *Pandalus jordani* and because the elution profiles of all the pigment-dispersing activities were the same he suggested that (a) a single substance activates more than one chromatophore type or (b) different but closely related molecules control each chromatophore type. However, when the pigment-dispersing activities of fractions of abdominal nerve cords are compared with those of eyestalk extracts as was done herein (Figs. 1 and 2) it becomes clear that at least two substances are involved. The eyestalk fractions evoked larger melanin-dispersing SIR's (particularly fractions 7–11) in the fiddler crab than red or white pigment-dispersing SIR's in the prawn (Fig. 1), but when the fractions of the abdominal nerve cords were assayed (Fig. 2) all of the fractions containing pigment-dispersing activities showed a greater effect on the prawn's leucophores than on the crab's melanophores and only one fraction evoked a larger SIR in the crab than it did with the erythrophores of the prawn. These differences among the relative overall melanin-dispersing and red and white pigment-dispersing potencies of the several fractions from the eyestalks and abdominal nerve cords would not have occurred if all these pigment-dispersing responses were due to a single substance. On the basis of these results it is clear that the eyestalks contain more of the melanin-dispersing substance and less of the red and white pigment-dispersing activities than do the abdominal nerve cords. The data with crude extracts (Table I) support the results shown in Figures 1 and 2 with respect to the relative quantities of the melanin-dispersing and red pigment-dispersing substances in the eyestalks and abdominal nerve cords. However, it was possible that there was an antagonism between the substances controlling dispersion and concentration of each pigment which could result in a much smaller response, or no response at all, than would occur if each substance was assayed in the absence of its antagonist. Therefore, it was necessary to filter these extracts through a gel known to separate pigment-dispersing and pigment-concentrating substances from each other (Fingerman, Bartell and Krasnow, 1971; Fingerman and Fingerman, 1972) before a definitive conclusion could be arrived at concerning the identity or non-identity of these pigment-dispersing substances.

The large amount of melanin-dispersing substance in the eyestalks of the prawn, which lacks melanophores, is enigmatic unless, of course, this substance has a different role in the prawn itself. The data presented above support the hypothesis that the role of this melanin-dispersing substance in the prawn is that of the hormone that causes light-adaptation of the distal retinal pigment for the following reasons. The elution patterns of the melanin-dispersing and distal retinal pigment light-adapting activities from both the eyestalks and abdominal nerve cords are the same (Figs. 1, 2, and 4). This similarity was also noted with extracts of eyestalks from the prawn *Pandalus jordani* (Kleinholz, 1970) and the fiddler crab *Uca pugilator* (Fielder, Rao and Fingerman, 1971). Furthermore, with both crude extracts and these partially purified fractions the eyestalks were more potent than the abdominal nerve cords with respect to both the melanin-dispersing and distal retinal pigment light-adapting activities. These fractions with distal retinal pigment light-adapting activity would be devoid of the dark-adapting hormone. Fingerman, Krasnow and Fingerman (1971) found that the latter was eluted much later than the former from Bio-Gel P-6, just as the pigment-concentrating material was

separated from the pigment-dispersing activities by this gel. Finally, the relative amounts of both the melanin-dispersing and distal retinal pigment light-adapting activities in the eyestalks and abdominal nerve cords were the same. Analysis of the data presented above by use of the dosage-response curves of Figures 3 and 5 showed that fraction 10 of the abdominal nerve cords contained not only 0.3 as much of the melanin-dispersing substance as did the eyestalks, but also only 0.3 as much distal retinal pigment light-adapting material as well.

The conclusion of Brown (1935) that the hormonal control of the erythrophores in this prawn has to be different from that of the leucophores was referred to above. However, the present experiments have not allowed us to conclude whether the difference in their control is due to the hormones which regulate dispersion of the red and white pigments, their concentration, or perhaps both processes. By the same logic which Brown (1935) used to arrive at his conclusion about the control of the chromatophores in *Palaemonetes,* a similar conclusion can be drawn that the hormonal control of the erythrophores and distal retinal pigment of this prawn cannot be the same because the pigment migrations which occur within one of these effectors are independent of migrations going on in the other. As seen above, when prawns are on a white background their red chromatophoric pigment is maximally concentrated and when they are on a black background this pigment is nearly maximally dispersed, but the distal retinal pigment may occupy a position that approaches the dark-adapted one or the light-adapted position regardless of whether the red chromatophoric pigment is dispersed or concentrated. Furthermore, on the basis of the present experiments we can conclude that one of the differences between the hormonal controls of each of these pigmentary effectors is that dispersion of the red and white chromatophoric pigments of *Palaemonetes* cannot be due to the substance which causes light adaptation of its distal retinal pigment. Whereas the eyestalk fractions were overall more potent than those of the abdominal nerve cords in evoking light adaptation of the distal retinal pigment (Fig. 4), the situation was reversed in the assays for chromatophoric pigment-dispersing activities in the prawn (Figs. 1 and 2).

Sandeen and Brown (1952) found that the distal retinal pigment of *Palaemonetes* responds to the brightness of the visual field (not an albedo response) whereas the responses of its chromatophores to black and to white backgrounds are a true albedo response. The observation herein that at each of the two light intensities used the mean DPI of the prawns in the black pans was lower than the mean DPI of the prawns in the corresponding white containers is in conformity with the results of Sandeen and Brown. Fingerman, Krasnow and Fingerman (1971) had suggested that this difference in response to illumination (albedo *versus* brightness of the visual field) by the chromatophores and distal retinal pigment of *Palaemonetes* respectively might be due to an underlying difference in their hormonal controls. The experiments described above revealed that such a difference does indeed exist.

We wish to thank the members of the Supply Department of the Marine Biological Laboratory for collecting the prawns and Miss Deborah K. Mobberly for her technical assistance.

Summary

1. Extracts of the eyestalks and abdominal nerve cords of the prawn *Palaemonetes vulgaris* were chromatographed on Bio-Gel P-6. The fractions of both extracts revealed melanin-dispersing activity in the fiddler crab *Uca pugilator* and red and white pigment-dispersing activities and distal retinal pigment light-adapting activity in the prawn. In addition, the eyestalk fractions contained red and white pigment-concentrating activities whereas only the red pigment-concentrating activity was found in the fractions of the abdominal nerve cords.

2. The pigment-dispersing and distal retinal pigment light-adapting activities were eluted from the column ahead of the pigment-concentrating activities and were consequently separated from them. However, the pigment-dispersing and distal retinal pigment light-adapting activities did not separate from each other. The pigment-concentrating activities likewise did not separate from each other.

3. The substance from the prawn which evokes melanin dispersion in the fiddler crab is not the substance that causes red pigment dispersion and perhaps white pigment dispersion as well in the prawn itself.

4. Furthermore, dispersion of the red and white chromatophoric pigments in the prawn is not caused by the substance that evokes light adaptation of its distal retinal pigment.

5. Evidence is presented to support the hypothesis that light-adaptation of the distal retinal pigment in *Palaemonetes* is caused by the substance found in the prawn that causes melanin dispersion in the fiddler crab. *Palaemonetes* itself lacks melanophores.

LITERATURE CITED

Brown, F. A., Jr., 1935. Control of pigment migration within the chromatophores of *Palaemonetes vulgaris*. *J. Exp. Zool.*, **71**: 1–15.

Brown, F. A., Jr., 1940. The crustacean sinus gland and chromatophore activation. *Physiol. Zool.*, **13**: 343–355.

Brown, F. A., Jr., and H. H. Scudamore, 1940. Differentiation of two principles from the crustacean sinus gland. *J. Cell. Comp. Physiol.*, **15**: 103–119.

Brown, F. A., Jr., H. M. Webb and M. I. Sandeen, 1952. The action of two hormones regulating the red chromatophores of *Palaemonetes*. *J. Exp. Zool.*, **120**: 391–420.

Fielder, D. R., K. R. Rao and M. Fingerman, 1971. Control of distal retinal pigment migration in the fiddler crab, *Uca pugilator*. *Mar. Biol.*, **9**: 219–223.

Fingerman, M., 1970. Dual control of the leucophores in the prawn, *Palaemonetes vulgaris*, by pigment-dispersing and pigment-concentrating substances. *Biol. Bull.*, **138**: 26–34.

Fingerman, M., C. K. Bartell and R. A. Krasnow, 1971. Comparison of chromatophorotropins from the horseshoe crab, *Limulus polyphemus*, and the fiddler crab, *Uca pugilator*. *Biol. Bull.*, **140**: 376–388.

Fingerman, M., and E. F. Couch, 1967. Differentiation of chromatophorotropins from the prawn, *Palaemonetes vulgaris*, and the fiddler crab, *Uca pugilator*. *J. Exp. Zool.*, **165**: 183–194.

Fingerman, M., and S. W. Fingerman, 1972. Evidence for a substance in the eyestalks of brachyurans that darkens the shrimp *Crangon septemspinosa*. *Comp. Biochem. Physiol.*, **43A**: 37–46.

Fingerman, M., R. A. Krasnow and S. W. Fingerman, 1971. Separation, assay, and properties of the distal retinal pigment light-adapting and dark-adapting hormones in the eyestalks of the prawn *Palaemonetes vulgaris*. *Physiol. Zool.*, **44**: 119–128.

FINGERMAN, M., K. R. RAO AND C. K. BARTELL, 1967. A proposed uniform method of reporting response values for crustacean chromatophorotropins: the Standard Integrated Response. *Experientia,* **23**: 962.

HOGBEN, L., AND D. SLOME, 1931. The pigmentary effector system.. VI. The dual character of endocrine co-ordination in amphibian colour change. *Proc. Roy. Soc. London, Series B,* **108**: 10–53.

KLEINHOLZ, L. H., 1936. Crustacean eye-stalk hormone and retinal pigment migration. *Biol. Bull.,* **70**: 159–184.

KLEINHOLZ, L. H., 1970. A progress report on the separation and purification of crustacean neurosecretory pigmentary effector hormones. *Gen. Comp. Endocrinol.,* **14**: 578–588.

PANTIN, C. F. A., 1934. The excitation of crustacean muscle. *J. Exp. Biol.,* **11**: 11–27.

PERKINS, E. B., 1928. Color changes in crustaceans, especially in *Palaemonetes. J. Exp. Zool.,* **50**: 71–105.

SANDEEN, M. I., AND F. A. BROWN, JR., 1952. Responses of the distal retinal pigment of *Palaemonetes* to illumination. *Physiol. Zool.,* **25**: 222–230.